Error in Back-Calculation of Lengths of Juvenile Southern Flounder, *Paralichthys lethostigma*, and Implications for Analysis of Size-Selection

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ABSTRACT: Otolith-based back-calculation methods should be useful for testing mechanisms that contribute to size-structure development in fish populations, such as size-based dispersal or habitat-dependent growth. We evaluated otolith-based back-calculation methods for testing the magnitude of size-dependent loss rate of juvenile southern flounder, *Paralichthys lethostigma*, from estuarine creek habitats. Periodicity of otolith increment formation was validated by sampling calcein-tagged juvenile flounder from an outdoor tank over a 106-d interval. In a second 2-mo rearing trial, juveniles were individually marked in order to estimate the error in back-calculation. A size hierarchy developed, with growth rates ranging from 0.08 mm d^{-1} to 1.4 mm d^{-1}, comparable to the variability observed from juveniles sampled across an estuarine depth gradient. Mean back-calculation errors based on linear regression for 1-mo and 2-mo intervals were estimated to be 8% and 5%, respectively. From analysis of variance (ANOVA) simulations, a shift of at least 10% between the mean of an observed length distribution and a back-calculated distribution (e.g., mimicking size-dependent removal of larger individuals) was detected, with 0.9 statistical power. However, power declined as back-calculation uncertainty was incorporated into the simulations. The magnitude of the shift in the mean can be related to instantaneous loss rate as a function of size, to provide a quantitative measure of the magnitude of size-selection.

Introduction

Southern flounder, *Paralichthys lethostigma*, is important as both a sport and commercial species in North Carolina. Juvenile southern flounder exhibit highly variable growth rates, ranging from 0.35 mm d^{-1} to greater than 1.5 mm d^{-1}, during the first summer. This leads to a bimodal length distribution by September of the first year (Fitzhugh 1993). In addition, larger age-0 flounders occupy differentially deeper water over an estuarine depth gradient (Weinstein 1979; Rogers et al. 1984; Fitzhugh 1993). This suggests that habitat or environmental effects on growth may be easily confused with size-dependent dispersal.

Events during the early life stage can determine key population attributes such as relative abundance of an age-class (Methot 1983). By examining the growth histories of young fish and the attributes of survivors over time, critical processes can be revealed (Rice et al. 1987; Pepin 1989). For instance, one hypothesis is that individuals growing faster during the larval and juvenile stage are more likely to contribute to the adult population (e.g., Peterson and Wroblewski 1984; Smith 1985; Pepin 1989). However, the general applicability of the "bigger is better" paradigm has been questioned (Litvak and Leggett 1992; Pepin et al. 1992). The analysis of otoliths offers a useful tool for critical tests of population-dynamic mechanisms (Campana and Neilson 1985; Rice et al. 1987; Pepin 1989). This approach is particularly useful for fishes that exhibit high growth variability during the first year, such as southern flounder (Gilbert 1986; Fitzhugh 1993). Variability in growth rates, leading to differential size-at-age, is a key ingredient for size-selective processes to operate with any intensity (Ricker 1969; Pepin 1989; Crowder et al. 1992; Rice et al. 1993).

We evaluated otolith-based back-calculation methods for testing the magnitude of size-dependent loss rate of juvenile southern flounder from estuarine creek habitats. These losses may reflect the dispersal or mortality of individuals as a function of size. To provide a basis for back-calculations of length distributions, we validated otolith increment periodicity and estimated back-calculation errors for 1-mo and 2-mo intervals. We then calculated the statistical power to detect particular magnitudes of shifts in the mean of a size distribution. For a given magnitude of change, we estimated the size-dependent loss rate necessary to effect such a shift.

Materials and Methods

We collected juvenile southern flounder by trawling in tributary creeks and along the margins of Pamlico Sound and Pamlico and Neuse rivers, North Carolina. Juveniles were collected near the size where piscivory is common (median total length (TL) approximately 100 mm; Powell and Schwartz 1979), and acclimated to feeding in holding tanks by first introducing small prey fishes (sciaenids, killifishes, anchovies) and, later, prepared food (frozen and thawed fish). Once they were actively feeding, flounders were fed to excess daily during rearing trials.

Two rearing trials were undertaken in a 4,000-l outdoor tank with a flow-through water system maintained at ambient temperatures (range 20-31°C). The first rearing trial was from June 7 to September 21, 1990 (106 d). It was used to establish the periodicity of increment formation in sagittae. Salinity during the 1990 trial ranged from 10‰ to 30‰. Prior to being placed into the outdoor tank, the flounders were immersed in a solution of 264 mg l^{-1} calcein (Sigma Chemical) for 24 h (Wilson et al. 1987) to incorporate a fluorescent mark on their otoliths. Subsamples of these flounders were taken at day 36 (July 13, 1990), day 70 (August 8, 1990), and at day 106 (September 21, 1990). We killed the flounders in a solution of MS-222. The samples were preserved frozen prior to processing. Later, the flounders were weighed, measured (mm TL), sexed, and their sagittal otoliths were removed and stored dry.

A second rearing trial was conducted from July 30 to October 5, 1991, to estimate the error in back-calculations of size from ring counts. As before, juveniles were immersed in a calcein bath but were also marked with visible implant tags (Northwest Marine Technology, Inc., Shaw Island, Washington, USA) inserted subdermally below the mandible on the unpigmented surface, in order to identify and measure individuals during the trial. During the 1991 trial, salinity was maintained

between 31‰ and 34‰. Flounders were netted, anesthetized with MS-222, measured, and returned to the tank on day 0 and day 38. At the end of the trial (day 67), "fresh" lengths were taken and flounders were killed and processed similarly to the earlier trial.

All otoliths were embedded in an epoxide resin (Buehler), and thin transverse sections were made through the core of the sagitta with a Buehler low-speed saw. The sections were then glued to a glass slide with thermoplastic cement (3M Crystalbond) and ground down to the primordium by use of a series of aluminum-oxide polishing papers and alumina polish (Buehler). We polished both surfaces until the primordium could be found and rings could be easily distinguished in the frontal plane (see Secor et al. 1991).

Southern flounder sagittae are asymmetrical during juvenile growth and so by convention only left sagittae were used for measurements and back-calculations (Smith et al. 1981; Sogard 1991). Many flatfish species have accessory primordia (Campana 1984), possibly due to metamorphic transition and asymmetrical otolith growth; this can obscure the interpretation of increment counts in certain planes of view (Secor et al. 1991; Sogard 1991). Using transverse sections in this study eliminated potential problems associated with interpreting sagittal sections of otoliths having accessory primordia.

Otoliths were viewed with transmitted light under a compound microscope fitted with a 100X oil-immersion objective, polarizing filters, reflected-light fluorescence attachments, and an image analysis system (total magnification to 1,250X). Fluorescence of calcein-marked otoliths was detected by passing incident ultraviolet (UV) light through a blue filter cube (Olympus DMB) with excitation filter bands from 400 nm to 490 nm and barrier filter band pass >515 nm. A 455-nm interference filter (Olympus EY 455) was also used, which reduced auto-fluorescence and enhanced the visibility of the calcein mark. We counted rings and measured each otolith along a radius from the primordium following the groove of the sulcus to its intersection with a tangential reference line drawn along the proximal surface of the sagitta (Fig. 1). Five consecutive ring counts on each sectioned otolith were made and the median value recorded. Preliminary work indicated the median was stable after five counts. We regarded the median, in contrast to the mean, as the most appropriate estimator for aging fish as otoliths were sometimes difficult to read, resulting in skewed counts (e.g., Rice et al. 1985). Our emphasis was on obtaining the most accurate count possible rather than achieving precision in counts, which can be viewed as a measure of ease in otolith readability (Campana and Moksness 1991).

The median increment count was regressed on days following calcein immersion for otolith samples from the 1990 rearing trial. Student's t-test was used to determine if the increment periodicity was daily (i.e., slope not significantly different from 1.0). We estimated the statistical power to detect a specified deviation from a slope of 1.0 at $p = 0.05$ level (two-sided test) (Rice 1987).

For back-calculations representing approximately 1-mo (29 d) and 2-mo (67 d) intervals, three counts were made proceeding inward from the otolith margin to locate position O_i (Fig. 1). Otoliths were then measured from the primordium to O_i, and the average distance calculated. Our back-calculation procedure follows Campana (1990) for application of simple linear regression, Fraser-Lee, and biological intercept methods. The linear-regression method requires an established proportionality of fish length and otolith size. The size of the fish at an earlier age is then estimated based on back-calculated increments and otolith size. The Fraser-Lee method is intercept-corrected based on the fish-otolith regression, and any individual deviations from the linear regression are maintained proportionally in back-calculation. The biological intercept method
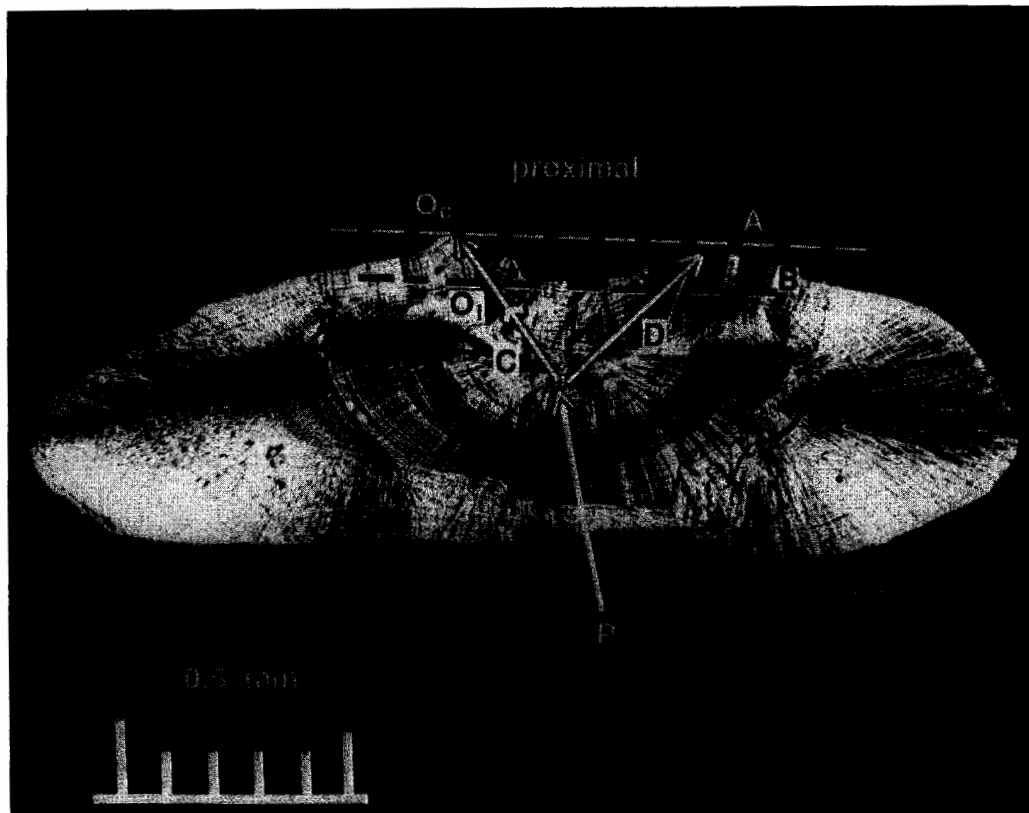
Fig. 1. Transverse section, viewing frontal plane of a sagitta from a 62-mm southern flounder (see Pannella 1980). A and B are reference lines tangent to proximal surface. O_c is the otolith radius at capture. O_i is the back-calculated otolith radius (location determined by ring count). P is the primordium. C is the line of radius measurement along the longest "wing" and denotes zone of increment counts. Ring counts (but not measurements) were also made across the shortest "wing" if necessary for age estimation (D-line).

eliminates the biases associated with a statistically derived intercept (used in the previous two methods), and instead uses an intercept based on actual measurement of some "small" fish length and otolith size. Multiple regressions, which were performed with SAS (SAS Institute Inc. 1987), incorporated growth rate as well as otolith size to predict fish length. Inverse regression was used to compute 95% confidence intervals of days given ring counts of 29 d and 67 d approximating 1-mo and 2-mo intervals, respectively (Draper and Smith 1981; Rice 1987).

Four small postsettlement juveniles (range 17-20 mm TL) were sampled April 19, 1989, and used to provide a mean biological intercept for the otolith-length relationship. Campana (1990) suggested the use of larval fish near the time of hatching for biological intercepts. However,

because this study was used to draw inferences about processes from larger juveniles and relies upon transversely sectioned otoliths, smaller individuals could not be used. These flounders are about the smallest size that possess sagittae large enough to mount, embed, and section in a transverse plane.

Hypothetical distributions and Monte Carlo simulations were used to estimate the statistical power to detect shifts in size-frequencies. Power is defined as the probability of correctly rejecting the null hypothesis (Peterman 1990). Our null hypothesis is that there is no difference between a measured and a back-calculated distribution, inferring that selectivity has no effect on a field distribution. Measurement of deviations in back-calculated versus actual measured length distributions can provide an estimate of the magnitude of size-dependent processes. First, we simulated a field distribution: a null distribution with the mean and variance taken from subsampled field data. We used an arbitrary subsample of flounder lengths from a larger dataset of flounder captured from North Carolina nursery creeks in July (n = 53), and computed the mean length (76.4 mm TL) and sample standard deviation (SD = 9.6) for this example. A hypothetical back-calculated distribution for a sample size of 30 was generated with a Monte Carlo procedure using this field mean and standard deviation. We tested shifts of 0%, 2%, 5%, 8%, 10%, 12%, and 15% in the mean of this field distribution, mimicking size-selective events. Next, uncertainty was incorporated into the back-calculated distribution (mimicking back-calculation error) by letting individual length values vary by a constant amount around their initial length. The incorporation of uncertainty (i.e., back-calculation error) into size distributions, decreases our ability to detect shifts in the mean of a distribution. Analysis of variance (ANOVA) simulations were used to evaluate the tendency for uncertainty to reduce the power to detect significant differences. One hundred consecutive ANOVAs were run to estimate the power needed to detect differences in the mean for a specified sample size and variance. The power to detect these shifts was estimated using Monte Carlo simulations of two-way ANOVA with SAS (1987). Our calculation of power was based on the proportion of the ANOVA runs (out of 100) where the null hypothesis (no difference between the two distributions) was rejected. Power curves were fit to plotted points from simulation output. This allowed us to determine the effectiveness of back-calculation under expected size-frequency shifts and sample-size constraints for a population of juvenile southern flounder (e.g., Peterman and Routledge 1983; Peterman 1990).

Size-frequency shifts were interpreted based on the method of Jones (1958) and Ricker (1969). These investigators estimated the degree of size-selective mortality necessary to account for a shift in the mean of a back-calculated distribution. For a lower-tail shift of a back-calculated distribution relative to a measured distribution (e.g., reflecting higher mortality of the largest individuals), the rate of increase in instantaneous loss rate per unit increase in fish length is

$$a = D/s^2$$

where D = decrease in mean length of the distribution and s = standard deviation in length of the measured distribution. This expression assumes that length distributions are normal and that instantaneous loss rate increases linearly with length. This function has been used to examine mortality and sampling bias (Jones 1958; Ricker 1969; West and Larkin 1987). In this paper, we use it to draw inferences about size-dependent loss rate, whether by dispersal, avoidance, or mortality.

Results

We interpreted otolith microstructure using terminology based on Pannella (1971, 1980) and Campana and Neilson (1985). Southern flounder sagittae grew beyond the sagittal plane during the juvenile stage. Transverse sections were required to accurately count increments and measure otolith radius in the same plane, which is necessary for back-calculation. Although transverse sections resulted in tight ring spacing from the primordium to the proximal edge, the approximate increment widths observed (1.5-2.5 μm) were well above the limit recommended for resolution with a light microscope (about 0.3 μm; Campana and Moksness 1991). Our theoretical resolution was about 0.2 μm based on our use of an oil-immersion lens with a numerical aperture of 1.3 (see Wischnitzer 1988).

During the increment-validation trial (1990), 37 juvenile flounders were marked with calcein (mean length = 77.9 mm TL, SD = 12.1) and placed into the raceway. Five flounders were removed on day 36, four on day 76, and six on day 106. Flounder mortality was greater than 50% and probably occurred near the beginning of the trial; we noted seven dead flounders (five were recovered) by day 3. The mean length for the five dead flounders was 61.9 mm TL (SD = 8.4), suggesting that smaller individuals were more susceptible to initial mortality. But, it was difficult to discern individual condition throughout the trial as the tank was large, contained natural sediment, and the flounders were cryptic, initially being detected only if startled. No other mortalities were observed during the trial. Mean lengths of sampled flounders were 85.2 mm TL (SD = 16.6) on day 36, 107.8 (SD = 11.3) on day 76, and 167.7 mm TL (SD = 18.6) on day 106, resulting in an estimated average growth rate of 0.85 mm d^{-1} based on survivors over the duration of the 1990 trial. We estimated the frequency of increment deposition by linear regression of median increment count on days following immersion in calcein (Fig. 2). The slope was not significantly different from 1 ($p > 0.05$, n = 15), indicating the periodicity of increments was approximately daily. The statistical power to detect a deviation of 0.1 from a slope of 1 was 0.60, and so provides reasonable confidence in the assumption of daily ring periodicity. A deviation of 0.15 from a slope of 1 could be detected with 0.93 power.

The second rearing trial (1991) was conducted to estimate back-calculation error. We immersed 16 juvenile flounders (mean length 99.4 mm TL, SD = 12.7) in calcein and tagged each with a visible implant tag prior to release in the raceway on July 7, 1991. One fish died on the day following release. After 38 d the raceway was drained, and the 15 remaining flounders (mean length = 137.8 mm TL, SD = 28.5) were recovered, measured, and returned to the raceway. Again, we noted one mortality the following day, a 117-mm individual. After an additional 29 d, the tank was drained for the final time, and the 12 remaining flounders (mean length = 175.8 mm TL, SD = 36.1) were recovered. Two flounders were missing from the final sample, but we had measured them on the second date; these individuals grew little over the first interval. The estimated mean growth rate for surviving flounders (mean of individual trajectories) over the duration of the trial was 1.08 mm d^{-1} (SD = 0.4). Only one individual surviving the duration of the trial did not grow (0.082 mm d^{-1}). Excluding this individual, the average growth rate of the flounders in the 1991 trial was 1.18 mm d^{-1} (SD = 0.26).

We examined the relationship between otolith radius and length via regression of natural log-transformed data, which indicated a one-to-one relationship (slope = 1.05, r^2 = 0.832) (Fig. 3). This relationship provides the intercept necessary for the Fraser-Lee method and confirmed the assumption of linearity for the biological intercept method (Table 1). The variance of the otolith
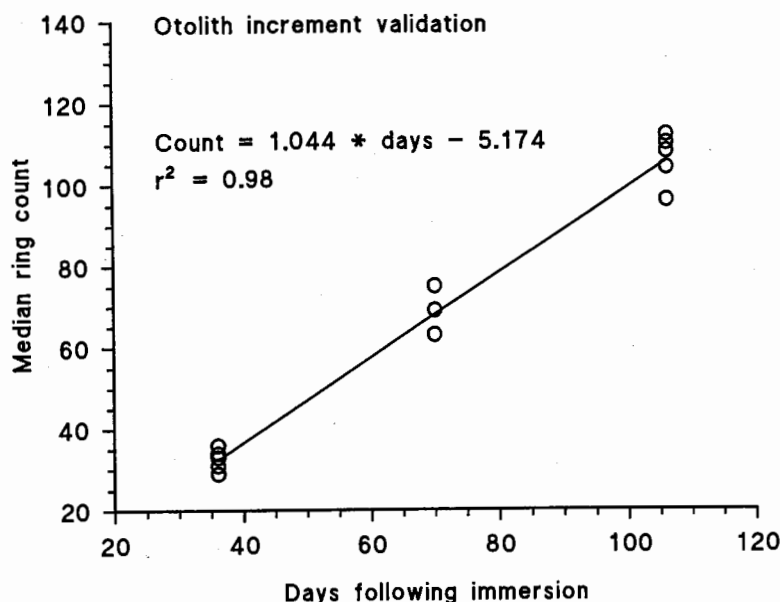
Fig. 2. Relationship of otolith ring count to days following immersion in calcein (fluorescent mark) in 1990 rearing trial. Linear regression to validate ring periodicity is indicated.

size-body length relationship increased with increasing otolith size, but was not normalized by log transformation (Fig. 3). This increase in variance, or decoupling of the body size to otolith size relationship, has been correlated to variable somatic growth rates (Resnick et al. 1989; Secor and Dean 1989; Campana 1990). Multiple regression incorporating both growth rate (G, mm d^{-1}, determined from otolith increment counts) and otolith radius (OR, μm) improved the prediction of total length (L, mm):

$$L = -43.095 + 0.219\,OR + 114.980\,G, \; r^2 = 0.96$$

and was also used to compute back-calculated sizes from marked flounders (Table 1).

Back-calculated lengths were compared with original measured lengths of tagged individuals from the 1991 rearing trial (Table 2). Only 10 of the 12 flounders surviving to the end of the trial were used in the multiple regression analysis; growth rate estimates necessary for regression could not be obtained for the remaining two fish due to the difficulty in increment interpretation in the nucleus. One individual survived half of the trial and was used for linear regression, Fraser-Lee, and biological intercept error estimates from back-calculations to the beginning of the trial (Table 2). Back-calculation error was lowest for the multiple-regression function for the last 29 d of the trial (6.5%), followed by linear regression (7.8%) for the same period (Table 2). Back-calculation error increased when we projected lengths back to the onset of the trial (67 d) based on all functions except linear regression, which decreased to 5.4% mean error (Table 2).
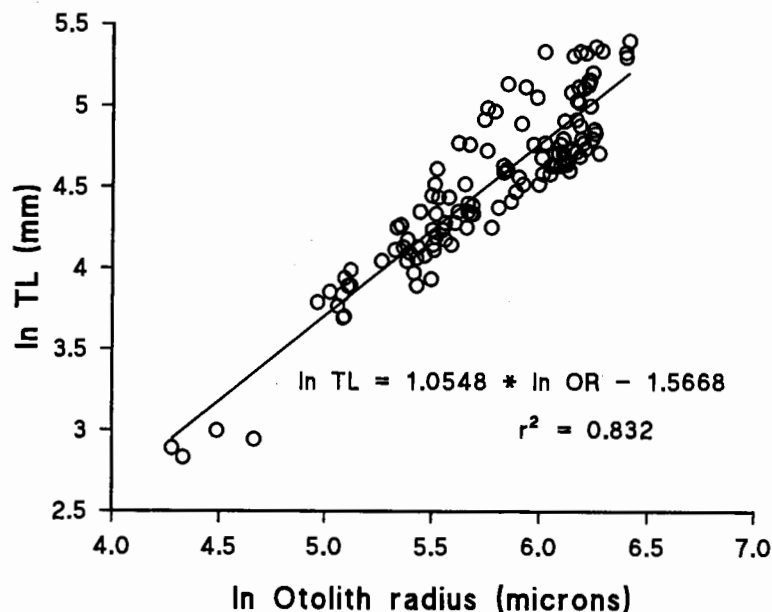
Fig. 3. Log-transformed (base e) relationship of otolith radius (line C in Fig. 1) to total length for flounders collected from the Pamlico Estuary, North Carolina.

We also projected back-calculations to the beginning of the trial using measurements to the calcein mark, to provide a estimate of back-calculation ability independent of counting error. Mean percent errors based on calcein marks compared to counts (67 d) were very similar for all back-calculation functions except linear regression, where error increased for calcein-marked individuals (10.5% for 67 d B-C) (Table 2). Inverse regression of ring counts indicated that 29 counts resulted in a mean of 32.7 d ± 4.2 (95% confidence interval, CI), and 67 counts represented a mean of 69.1 d ± 2.6 (95% CI). This agreement between calcein-marked and count-determined results suggests that back-calculation error was not appreciably affected by increasing uncertainty in ring counts over the interval examined. However, this does not eliminate variation in the fish length-otolith size relationship as a source of error.

The residuals indicated that back-calculation errors were not randomly distributed (Figs. 4 and 5). For simple linear regression, residuals increased with observed length for the 29-d interval, indicating the predicted lengths were underestimated (Fig. 4a). However, for linear regression over 67 d (back to the beginning of the trial), there was no clear pattern of bias in the residuals (Fig. 5a). Multiple regression also showed no pattern of bias of the residuals for back-calculation to the midpoint of the 1991 rearing trial (Fig. 4b). Residuals for other back-calculation methods showed a tendency to be negative, indicating an overestimation of sizes via back-calculation (Figs. 4 and 5).

The variance in flounder length increased during the trials. During the 1991 trial, the coefficient of variation for length increased from 0.13 to 0.21. The otolith size-length relationship

Table 1. Functions used in back-calculation; L_i = back-calculated length (TL, mm), L_c = length at capture, L_o = length at origin for biological intercept, O_c = otolith radius at capture (units = μm), O_i = back-calculated otolith radius, O_o = biological intercept (origin) for otolith intercept, G = growth rate (L_c/ring count), b and c are coefficients derived from fit of equations, and d is the fitted intercept (see Campana 1990).

Function	d
Simple Linear Regression $L_i = \exp[b\,(\ln O_i) + d]$	−1.567
Multiple Regression $L_i = d + bO_i + cG$	−43.095
Biological Intercept[1] $L_i = \exp[\ln L_c + ((\ln O_i - \ln O_c)(\ln L_c - \ln L_o)(\ln O_c - \ln O_o)^{-1})]$	—
Fraser-Lee $L_i = \exp[d + ((\ln L_c - d)(\ln O_c)^{-1}(\ln O_i))]$	−1.567

[1]$\ln L_o = 2.89$, $\ln O_o = 4.279$

Table 2. Back-calculation errors determined from individually marked flounder (1991 rearing trial). Back-calculations were made for 29-d and 67-d periods when length measurements were taken during the trial. Back-calculation to the calcein mark was computed from measurements from the primordium to the mark (not based on ring counts).

|	n	mean % error	SD of mean
Trial midpoint (29 d)			
Simple linear regression	12	7.8	5.4
Multiple regression	10	6.5	4.3
Biological intercept	12	15.3	9.2
Fraser-Lee	12	16.1	3.8
Beginning of trial (67 d)			
Simple linear regression	13	5.4	5.3
Multiple regression	10	26.0	11.9
Biological intercept	13	21.5	10.6
Fraser-Lee	13	24.5	28.5
Calcein mark			
Simple linear regression	12	10.5	5.3
Multiple regression	10	26.0	11.9
Biological intercept	12	22.1	10.6
Fraser-Lee	12	25.1	28.5

Back–calculation to midpoint of trial
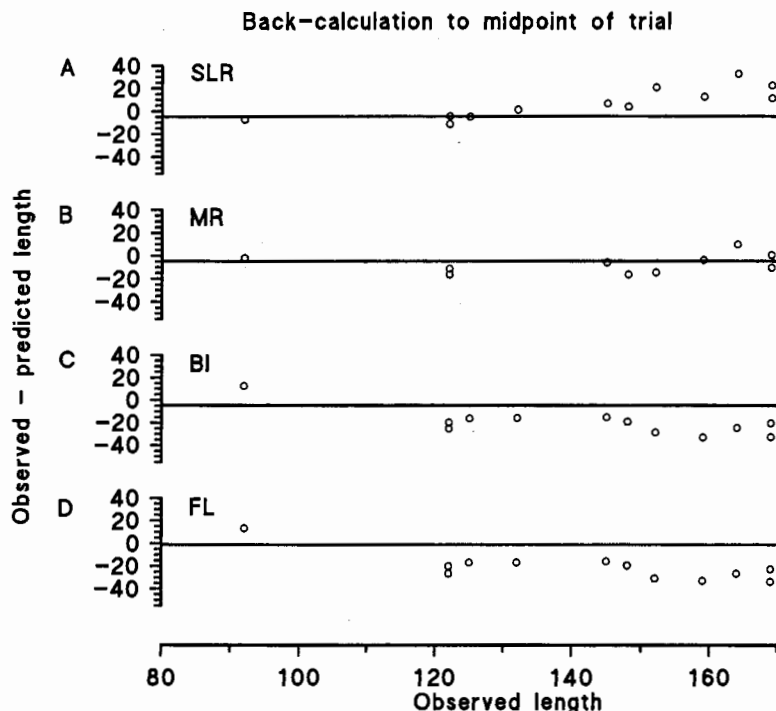
Fig. 4. Residuals from back-calculated lengths compared to observed lengths from the midpoint in the 1991 rearing trial. Predicted lengths were calculated using simple linear regression (SLR), multiple regression (MR), biological intercept (BI) and Fraser-Lee (FL) methods.

for the 1991 trial where flounders were individually tagged was uncoupled, that is, for each individual, the relationship between fish size and otolith size was not constant (Fig. 6). Most of the individuals grew at rates >1 mm d^{-1}, and their body length increased more rapidly than their otolith radius. The single surviving individual that did not grow fell below the one-to-one line of the somatic length-otolith size relationship.

When we used ANOVA to examine the power to detect shifts in back-calculated distributions, there were strong effects of back-calculation uncertainty on power. When there was zero variation (SD = 0) about randomly drawn individual values (i.e., perfect back-calculation), a shift from 76.4 mm to 70 mm mean length was detected with an approximate power of 85% (Fig. 7). When individual values for length were varied by standard deviations of 6 and 12 (i.e., back-calculation errors of approximately 8% and 15%, respectively, estimating the standard deviation as one half the range of observed values; Snedecor and Cochran 1967), power declined to about 75% and 65%, respectively (Fig. 7).

The example above was based on a hypothetical back-calculated (shifted) normal distribution of sample size 30 (smaller sample sizes (< 30) would be better represented by a t-distribution; Snedecor and Cochran 1967), and deviations of just a few millimeters could only be detected with low power (20% to 30% for a 2-mm to 4-mm shift; Fig. 7). When the sample size for the back-
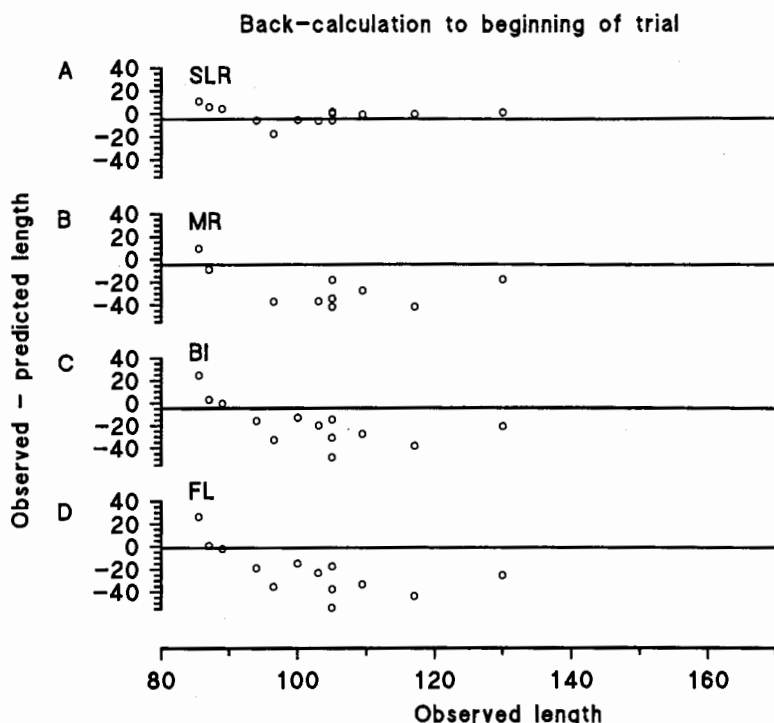
Back-calculation to beginning of trial

Fig. 5. Residuals from back-calculated lengths compared to observed lengths at the beginning of the 1991 rearing trial. Predicted lengths were calculated using simple linear regression (SLR), multiple regression (MR), biological intercept (BI) and Fraser-Lee (FL) methods.

calculated (shifted) distribution was increased to 100, the power to detect a 4-mm shift increased to 67%. When sample size was increased to 200, the power increased to 72%. Although these are only approximations of the statistical power to detect shifts in normal distributions, they suggest that increased effort (e.g., up to 100 flounder) would aid us in detection of small differences (i.e., on the order of 4-10 mm), which we expect will be required to make inferences about size-dependent dispersal.

Jones (1958) and Ricker (1969) found that an individual's per-unit increase in length can be related to a per-unit increase of instantaneous loss rate if size-selective removal is a positive linear function of length. The amount of deviation in a length distribution can be used to test for size-dependent events and can be related to the amount of change in instantaneous loss as a function of length. From the previous example, a shift in the distribution to a mean of 70 mm from 76.4 mm (D = 6.4, s = 9.6) would mean that for each 1-mm increase in length for a flounder there was a corresponding increase in instantaneous rate of removal of 0.069 (individuals per individual per day). Thus, individuals at the upper and lower tails of the measured length distribution (two standard deviations above and below the mean) would differ by 2.6 (i.e., 4sa) in their respective loss rate coefficients. This example corresponds to a loss of larger individuals at a higher rate than smaller individuals.
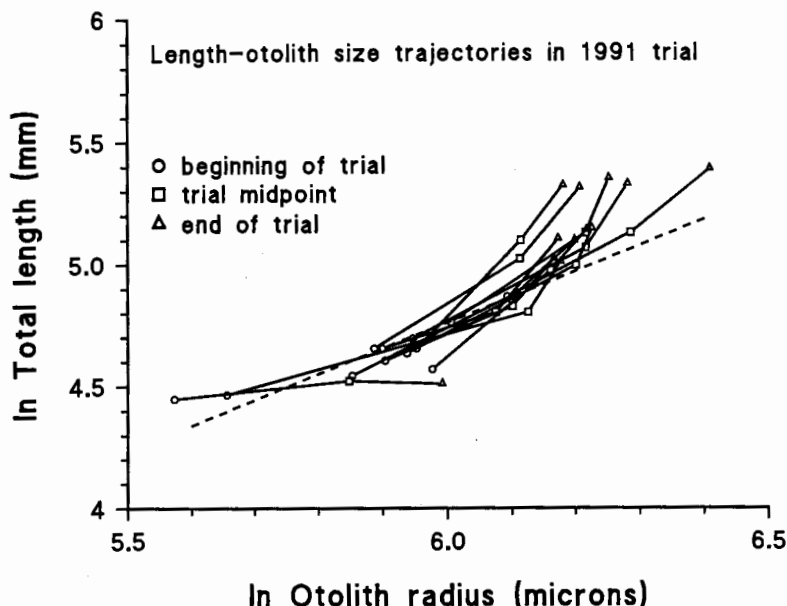
Fig. 6. Log-transformed (base e) relationship between length and otolith radius for individual growth trajectories from the 1991 rearing trial. Otolith radius was calculated from positions O_i for the beginning and midpoint of the trial and measurement O_c at the end of the trial (see Fig. 1). Dashed line is the regression fit from field-collected flounders (see Fig. 3).

Discussion

Use of otolith back-calculation methods offers much promise for detecting and quantifying critical population-dynamic processes such as size-selective mortality and movements (Wilson and Larkin 1982; Pepin 1989). However, necessary precursors in applying back-calculation procedures are otolith increment validation and establishing a fish size-otolith size relationship. Our otolith validation study revealed a strong, linear relationship between increment counts and days-at-large following calcein marking. We conclude that increments are daily. This daily periodicity was established well into the juvenile stage as we observed increment formation over 106 d following calcein marking of 3-mo-old to 5-mo-old flounders. The intercept in the increment count-days relationship was negative, revealing a consistent underestimate of about five rings during counts. The negative intercept may be due to poor resolution of rings on the edge of the otolith sections. Our use of a large (4,000 l) outdoor tank for evaluating otolith-based methods in back-calculation was a good compromise between field release of marked individuals, which suffers from a difficulty in recapturing and restrictions on use of chemical markers, and laboratory rearing in which aberrations in otolith patterns are common (Campana and Moksness 1991).
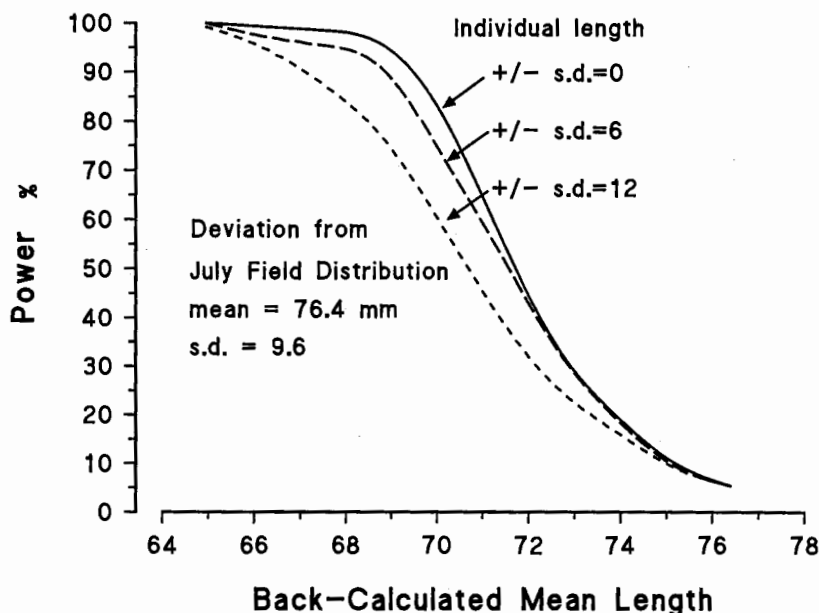
Fig. 7. Power curves for detecting shifts in a mean of a specified length distribution (mimicking July field distribution). Distributions were generated by randomly drawing 30 observations (lengths) with a specified mean and standard deviation (9.6 mm). Individual draws (lengths) were then allowed to vary by a standard deviation of 0 mm, 6 mm, and 12 mm (using each random draw as a mean) to mimic back-calculation uncertainty.

The patterns of growth exhibited by flounders during the rearing trials were typical of phenomena that have caused problems for back-calculation applications in previous studies (see Campana 1990). These problems include the violation of the assumption of linear growth trajectories, which is required by most back-calculation methods, and individual differences in the length-otolith size relationship. Theoretically, increment-width analysis could be applied to account for curvature in the length-otolith size relationship on the basis of summing daily back-calculated length increments (Campana 1990). But, in practice, application of this technique has proven difficult to implement and only marginally useful due to factors other than somatic growth that affect increment width (Resnick et al. 1989; Secor and Dean 1992).

We compared the more common back-calculation methods to estimate error in back-calculation and to determine the conditions under which we could make valid conclusions about size-dependent loss rates. Positive and negative departures from the one-to-one somatic length-otolith size relationship (O-L) resulted in biases when we back-calculated lengths and compared results with earlier measured lengths. Fraser-Lee and biological intercept methods base the back-calculation on an individual linear slope of the O-L relationship from an observed point at capture to an intercept (i.e., an intercept either statistically derived or determined from biological observation). For fast-growing flounders with a positive departure from the O-L relationship, back-calculated lengths were too large. In contrast, for a slow-growing flounder with a negative departure, back-calculated
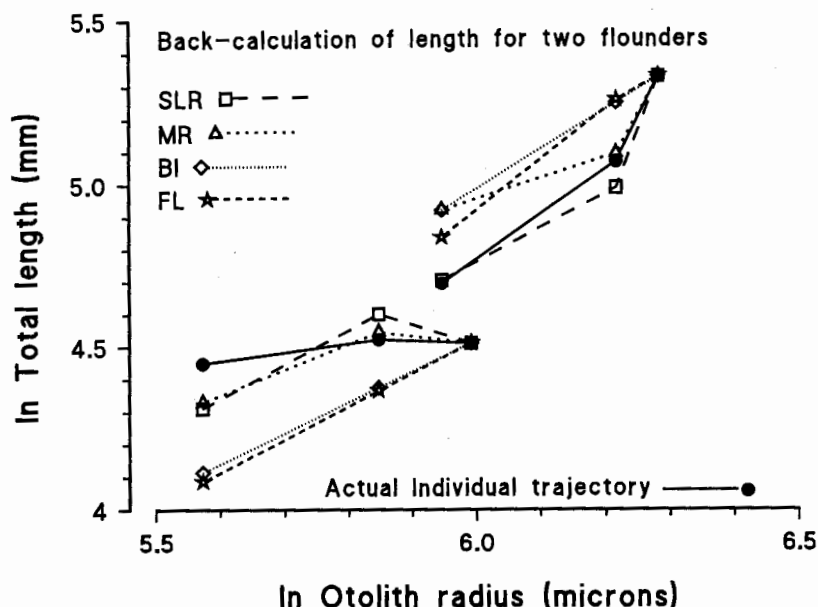
Fig. 8. Back-calculation trajectories including simple linear regression (SLR), multiple regression (MR), biological intercept (BI) and Fraser-Lee (FL) methods for two flounders (see Fig. 6): one fast-growing and one slow-growing flounder chosen from the 1991 rearing trial.

length was too small (Fig. 8). Therefore, the nonrandom bias (overestimation) observed for the Fraser-Lee and biological intercept residuals occurred because most of the flounders departed positively from the one-to-one O-L line. Patterns of bias in the residuals due to nonlinear growth have also been detected by Secor and Dean (1992) for back-calculated lengths of larval striped bass (*Morone saxatilis*).

Time-varying growth also affected back-calculations of size using the multiple regression technique. Multiple regression, which incorporates linear growth rate, was least biased at the trial midpoint, resulting in fairly accurate estimates of flounder length (Fig. 8). This was due to the rapid change in growth that occurred in the last half of the trial and affected the linear growth estimate. However, back-calculation using multiple regression to the beginning of the trial, when somatic growth was not changing rapidly relative to otolith growth, resulted in the same pattern of bias as observed for the Fraser-Lee and biological intercept methods due to the dependence on assuming a linear growth trajectory. Our growth rates were determined from the overall change in length of the survivors over the course of the trial. Therefore, when we incorporated growth into the multiple regression, we maintained a proportional error in back-calculation similar to the Fraser-Lee and biological intercept methods. Secor and Dean (1992) found that a quadratic term, incorporating age into the regression, improved the prediction of back-calculated size and performed better where other methods failed to account for periods of negative growth. The disadvantage of these more complex models is the requirement for unbiased estimates of age as well as length and otolith size (Secor and Dean 1992).

Back-calculating lengths by linear regression demonstrated the lowest error over the duration of the 1991 rearing trial. This apparently occurred because we were back-calculating length to a size prior to which the flounders departed from the O-L line. Previous discussions of back-calculation and application of linear regression has indicated that the population O-L relationship does not allow for any deviation of individual fish and otolith measurements; hence, the preference for the Fraser-Lee or biological intercept methods over the regression method (Carlander 1981; Campana 1990). Our problems of bias with the biological-intercept method may have been partly due to our small sample size and the ontogenetic stage of flounders (early juveniles rather than larvae), and may have contributed to an inappropriate intercept. However, linear regression has been most often applied when mean back-calculated lengths as opposed to individual estimates are of importance (Campana 1990). Further, the determination of the mean has been shown to be less prone to error than the estimation of individual values (Campana and Moksness 1991). If the desired application is to detect shifts in the mean of a distribution, use of linear regression in back-calculation is appropriate.

Are the levels of error in back-calculation great enough to suppress our ability to draw inferences about size-selection? The average error rates we found for linear regression, 8% and 5% for approximately 1 mo and 2 mo, respectively, are lower than findings reported by Wilson and Larkin (1982). They found that the error in back-calculation of weight over a 2-mo period was about 15% based on a linear relationship of otolith radius and weight of sockeye salmon (*Oncorhynchus nerka*) fry. Wilson and Larkin (1982) also noted a pattern in the residuals that may have been a result of the procedure of back-calculation; that is, fish with large weight-to-radius ratios were subject to overestimation in back-calculation while those with small weight-to-radius ratios were subject to underestimation. While they found this magnitude of error to be satisfactory for hypothesis testing of distribution shifts (see West and Larkin 1987), they relied on reading and measuring otoliths from the sagittal plane and felt that improvements in otolith preparation could remove some of the variation in radius measurements that occurred (i.e., on the order of ± 5%). From our results, the error in measurement was lower, ranging from 0.1% to 1.3% for radius measurements from cross-sectioned otoliths. Analysis of transverse sections on an image analysis system facilitated these measurements.

Given our estimate of error in back-calculation of the mean of a length distribution, we wished to know our ability to detect a shift in the mean. An a priori estimation of power to detect distribution shifts can strengthen inferences about the magnitude of size-selective processes and aid in estimating the necessary sample size (Peterman 1990). If the magnitude of the shift is expected to be large, sample size can be minimized and still maintain a desirable level of power (e.g., 80% or higher; Peterman 1990). If effects of size-selective processes are expected to be subtle, expected deviations from the mean of a length distribution will correspondingly be smaller, and so sample size will have to increase to maintain appropriate power. From the simulations it was evident that shifts in distributions of only a few millimeters could be detected with fair power (67% to 72%) only if the sample size was relatively high (i.e., 100-200 otoliths). Error in back-calculation has to be appreciable (greater than 15%) in the example given to reduce power by greater than 10-20%. However, when subtle size-selective effects are expected, efforts to reduce error such as more elaborate otolith preparation may be necessary.

Provided shifts in the mean of length frequency distributions can be detected by back-calculation methods, these shifts can be used to infer the magnitude of the size-selective loss rate. Jones (1958) and Ricker (1969) explored the consequences of back-calculating length distributions

when size-selection is occurring (e.g., due to predation or gear selection). Deviations of only a few millimeters in mean length of a juvenile size distribution could suggest quite a large change in size-selective processes, assuming a linear relationship between instantaneous rate of loss and length (Jones 1958; Ricker 1969). Lower tail shifts imply the largest individuals are being lost at a higher rate (positive size selection). This positive selection is typical of recruitment to a fishery, gear avoidance (Ricker 1969), and dispersal by larger individuals (e.g., away from a nursery area; Knudsen and Herke 1978). Negative size selection entails a shift in the mean toward the upper tail of a length distribution, and is typical of size-selective mortality (e.g., predation) operating on the smallest individuals (Ricker 1969; West and Larkin 1987).

If both negative and positive size-selection are occurring, their counteracting effects may lead to failure to reject the null hypothesis that size-selection does not exist. Before accepting the null hypothesis, it would be best to examine biological evidence directly. For example, if back-calculated shifts are not detected, but tag recaptures or trap samples are represented by the largest individuals in a locality, the evidence would suggest both size-selective movements and mortality are occurring and are offsetting each other.

Variability of the length distribution is not likely to be affected by size-selectivity as long as the change in instantaneous loss rate with length is linear (Jones 1958; Ricker 1969). Nonlinear selective mortality could cause variability in a length distribution to increase or decrease. However, even an extreme deviation from a linear relationship, such as a step function, has a small effect on the variability of a distribution (Ricker 1969). If variability of a length distribution remains relatively unaffected by size-selective removals, then detecting shifts in the mean of a back-calculated distribution is an appropriate approach for making inferences about size-selective processes.

Linear regression for back-calculation of lengths was the least biased method for our marking and growth trial. While we note its usefulness in our application, we also caution against over-interpretation of results regarding selectivity. Our field samples, used to fit the regression of length and otolith size, were dominated by sizes of flounders less than the size-at-piscivory. Similar to many of our field samples, we back-calculated lengths, using regression, to a stage when flounders were not readily piscivorous and individuals were about the same size. We believe this is a main reason why mean back-calculated length was closely correlated to mean measured length when we back-calculated to the beginning of the 1991 trial. Regression results can be dependent on the choice of field data, and undetected bias using a regression method may lead to misinterpretation of the mechanism and degree of selectivity (Carlander 1981; Campana 1990; Mosegaard 1990).

Because size-selective processes are likely to be important where variability in growth is relatively large among members of a population (Crowder et al. 1992; Rice et al. 1993), nonlinear growth patterns, which violate most back-calculation assumptions, may be common. How do we reconcile the use of these back-calculation methods with the possibility of nonlinear growth trajectories among individuals? Lacking a general model of otolith growth and somatic growth (Secor and Dean 1992), several approaches may enhance our ability to make inferences about selectivity, given the constraints of common back-calculation methods. First, we feel that our use of rearing trials aided us in understanding the mechanism and degree of growth variability (due to the onset of piscivory, in our example), and provided us an estimate of error in back-calculation. Based on this error, simulations to calculate power allow estimation of what magnitude of selectivity (as determined by distribution shifts) can be detected. This approach provides the measure of confidence in accepting the null hypothesis of no selection. Additionally, randomization

modeling of nonlinear growth (e.g., Campana 1990) and selectivity (e.g., Mosegaard 1990) enables an evaluation of the affects of back-calculation bias on the interpretation of selectivity.

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LITERATURE CITED

Campana, S.E. 1984. Microstructural growth patterns in the otoliths of larval and juvenile flounder, *Platichthys stellatus*. *Canadian Journal of Zoology* 62: 1507-1512.

Campana, S.E. 1990. How reliable are growth back-calculations based on otoliths? *Canadian Journal of Fisheries and Aquatic Science* 47: 2219-2227.

Campana S.E. and E. Moksness. 1991. Accuracy and precision of age and hatch date estimates from otolith microstructure examination. *International Council for the Exploration of the Sea, Journal of Marine Science* 48: 303-316.

Campana, S.E. and J.D. Neilson. 1985. Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Science* 42: 1014-1032.

Carlander, K.D. 1981. Caution on the use of the regression method of back-calculating lengths from scale measurements. *Fisheries* 6: 2-4.

Crowder, L.B., J.A. Rice, T.J. Miller, and E.A. Marshall. 1992. Empirical and theoretical approaches to size-based interactions and recruitment variability, p. 237-255. *In* D.L. DeAngelis (ed.), Individual-Based Approaches in Ecology. Routledge, Chapman and Hall, New York.

Draper, N.R. and H. Smith. 1981. Applied Regression Analysis. 2nd ed. John Wiley and Sons, New York.

Fitzhugh, G.R. 1993. An individual-based approach to understanding patterns of differential growth and population size structure in juvenile southern flounder (*Paralichthys lethostigma*). Ph.D. thesis, North Carolina State University, Raleigh, North Carolina.

Gilbert, C.R. 1986. Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (South Florida)--southern, gulf, and summer flounders. United States Fish and Wildlife Service Biological Report 82(11.54). United States Army Corps of Engineers, TR EL-82-4. 27 p.

Jones, R. 1958. Lee's phenomenon of "apparent change in growth rate," with particular reference to haddock and plaice. *International Commission for the Northwest Atlantic Fisheries Special Publication* 1: 229-242.

Knudsen, E.E. and W.H. Herke. 1978. Growth rate of marked juvenile Atlantic croakers, *Micropogon undulatus*, and length of stay in a coastal marsh nursery in southwest Louisiana. *Transactions of the American Fisheries Society* 107(1): 12-20.

Litvak, M.K. and W.C. Leggett. 1992. Age and size-selective predation on larval fishes: The bigger-is-better hypothesis revisited. *Marine Ecology Progress Series* 81: 13-24.

Methot, R.D. 1983. Seasonal variation in survival of northern anchovy estimated form the age distribution of juveniles. *Fishery Bulletin* 81: 741-750.

Mosegaard, H. 1990. What is reflected by otolith size at emergence? - A reevaluation of the results in West and Larkin (1987). *Canadian Journal of Fisheries and Aquatic Science* 47: 225-228.

Pannella, G. 1971. Fish otoliths: Daily growth layers and periodic patterns. *Science* 173: 1124-1127.

Pannella, G. 1980. Growth patterns in fish sagittae, p. 519-560. *In* D.C.Rhoads and R.A Lutz (eds.), Skeletal Growth of Aquatic Organisms. Plenum Press, New York.

Pepin, P. 1989. Using growth histories to estimate larval fish mortality rates. *Rapports et Proces-Verbaux des Reunions Conseil International pour l'Exploration de la Mer* 191: 324-329.

Pepin, P., T.H. Shears, and Y. de Lafontaine. 1992. Significance of body size to the interaction between a larval fish (*Mallotus villosus*) and a vertebrate predator (*Gasterosteus aculeatus*). *Marine Ecology Progress Series* 81: 1-12.

Peterman, R.M. and R.D. Routledge. 1983. Experimental management of Oregon coho salmon (*Oncorhynchus kisutch*): Designing for yield of information. *Canadian Journal of Fisheries and Aquatic Science* 40: 1212-1223.

Peterman, R.M. 1990. Statistical power analysis can improve fisheries research and management. *Canadian Journal of Fisheries and Aquatic Science* 47: 2-15.

Peterson, I. and J.S. Wroblewski. 1984. Mortality rate of fishes in the pelagic ecosystem. *Canadian Journal of Fisheries and Aquatic Science* 41: 117-1120.

Powell, A.B. and F.J. Schwartz. 1979. Food of *Paralichthys dentatus* and *P. lethostigma* (Pisces: Bothidae) in North Carolina estuaries. *Estuaries* 2: 276-279.

Resnick, D., E. Lindbeck, and H. Bryga. 1989. Slower growth results in larger otoliths: An experimental test with guppies (*Poecilia reticulata*). *Canadian Journal of Fisheries and Aquatic Science* 46: 108-112.

Rice, J.A. 1987. Reliability of age and growth-rate estimates derived from otolith analysis, p. 167-176. *In* R.C. Summerfelt and G.E. Hall (eds.), The Age and Growth of Fish. The Iowa State University Press, Ames, Iowa.

Rice, J.A., L.B. Crowder, and F.P. Binkowski. 1985. Evaluating otolith analysis for bloater *Coregonus hoyi*: Do otoliths ring true? *Transactions of the American Fisheries Society* 114: 532-539.

Rice, J.A., L.B. Crowder, and M.E. Holey. 1987. Exploration of mechanisms regulating larval survival in Lake Michigan bloater: A recruitment analysis based on characteristics of individual larvae. *Transactions of the American Fisheries Society* 116: 703-718.

Rice, J.A., T.J. Miller, K.A. Rose, L.B. Crowder, E.A. Marschall, A.S. Trebitz, and D.L. DeAngelis. 1993. Growth rate variation and larval survival: Inferences from an individual-based, size-dependent predation model. *Canadian Journal of Fisheries and Aquatic Science* 50: 133-142.

Ricker, W.E. 1969. Effects of size-selective mortality and sampling bias on estimates of growth, mortality, production, and yield. *Journal of the Fisheries Research Board of Canada* 26: 479-541.

Rogers, S.G., T.E. Targett, and S.B. Von Sant. 1984. Fish-nursery use in Georgia saltmarsh estuaries: The influence of springtime freshwater conditions. *Transactions of the American Fisheries Society* 113: 595-606.

SAS Institute Inc. 1987. SAS/STAT Guide for Personal Computers. Version 6 ed. SAS Institute Inc. Cary, North Carolina.

Secor, D.H. and J.M. Dean. 1989. Somatic growth effects on the otolith-fish size relationship in young pond-reared striped bass, *Morone saxatilis* (Walbaum). *Canadian Journal of Fisheries and Aquatic Science* 46: 113-121.

Secor, D.H. and J.M. Dean. 1992. Comparison of otolith-based back-calculation methods to determine individual growth histories of larval striped bass, *Morone saxatilis. Canadian Journal of Fisheries and Aquatic Sciences* 49: 1439-1454.

Secor, D.H., J.M. Dean, and E.H. Laban. 1991. Manual for Otolith Removal and Preparation for Microstructural Examination. Electric Power Research Institute and the Belle W. Baruch Institute for Marine Biology and Coastal Research, University of South Carolina, Columbia, South Carolina.

Smith, P. 1985. Year-class strength and survival of 0-group clupeoids. *Canadian Journal of Fisheries and Aquatic Science 42* (supplement 1): 69-82.

Smith, R.W., L.M. Dery, P.G. Scarlett, and A. Jerald, Jr. 1981. Proceedings of the Summer Flounder (*Paralichthys dentatus*) Age and Growth Workshop, 20-21 May 1980, Northeast Fisheries Center, Woods Hole, Massachusetts. National Oceanic and Atmospheric Administration Technical Memorandum NMFS-F/NEC-11.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical Methods. The Iowa State University Press. Ames, Iowa.

Sogard, S. M. 1991. Interpretation of otolith microstructure in juvenile winter flounder (*Pseudopleuronectes americanus*): Ontogenetic development, daily increment validation, and somatic growth relationships. *Canadian Journal of Fisheries and Aquatic Science* 48: 1862-1871.

Weinstein, M.P. 1979. Shallow marsh habitats as primary nurseries for fish and shellfish, Cape Fear River, North Carolina. *Fishery Bulletin* 77: 339-357.

West, C.J. and P.A. Larkin. 1987. Evidence for size-selective mortality of juvenile sockeye salmon (*Onchorhynchus nerka*) in Babine lake, British Columbia. *Canadian Journal of Fisheries and Aquatic Science* 44: 712-721.

Wilson, C.A., D.W. Beckman, and J.M. Dean. 1987. Calcein as a fluorescent marker of otoliths of larval and juvenile fish. *Transactions of the American Fisheries Society* 116: 668-670.

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Wilson, K.H. and P.A. Larkin. 1982. Relationship between thickness of daily growth increments in sagittae and change in body weight of sockeye salmon (*Oncorhynchus nerka*) fry. *Canadian Journal of Fisheries and Aquatic Science* 39(10): 1335-1339.
Wischnitzer, S. 1988. Introduction to Electron Microscopy. 3rd ed. Pergamon Press, Elmsford, New York.

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